

## Supplementary Information

### ***In Vitro* and *In Vivo* Evaluation of Novel Chromeno[2,3-*d*] pyrimidinones as Therapeutic Agents for Triple Negative Breast Cancer**

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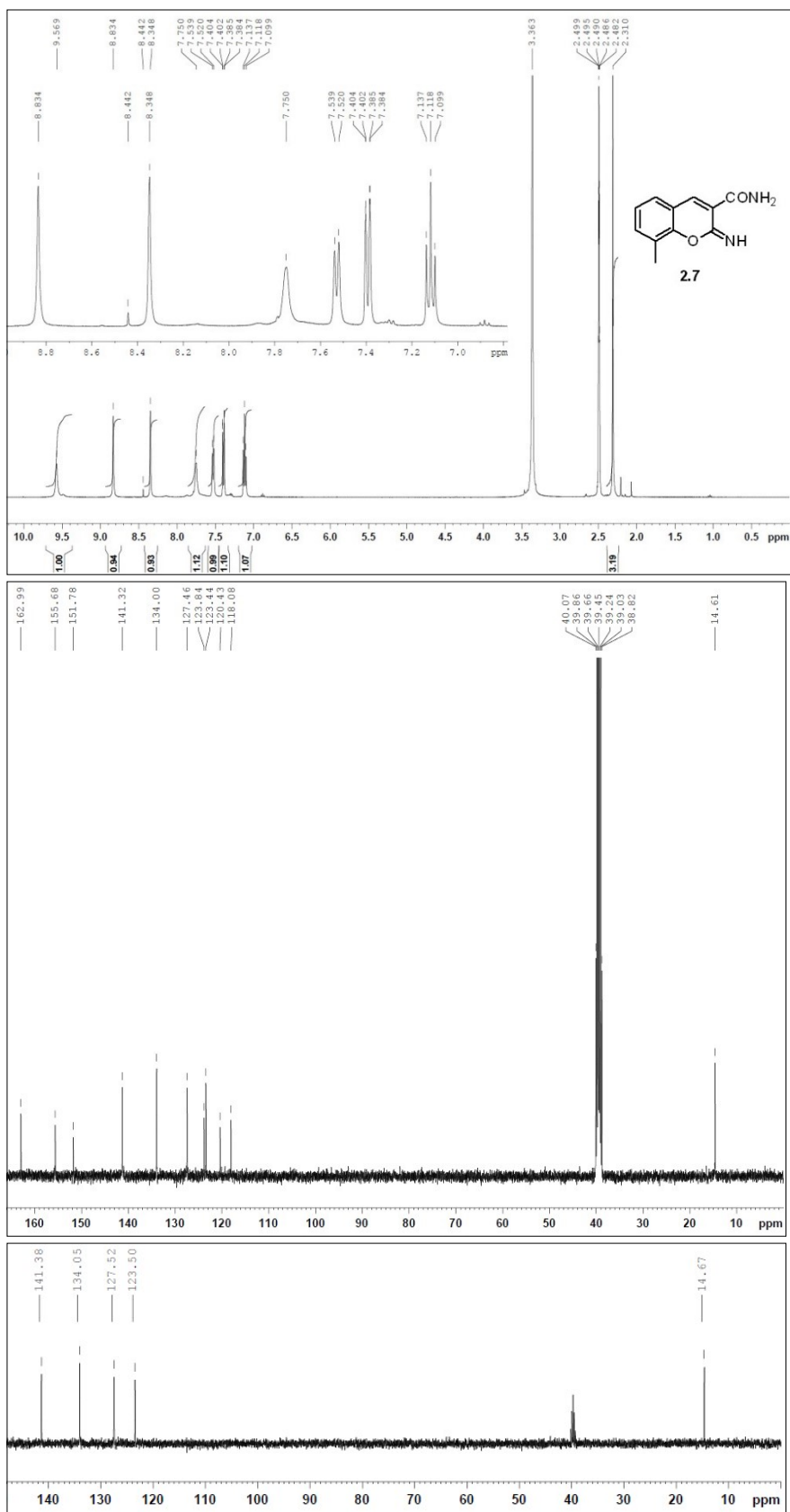
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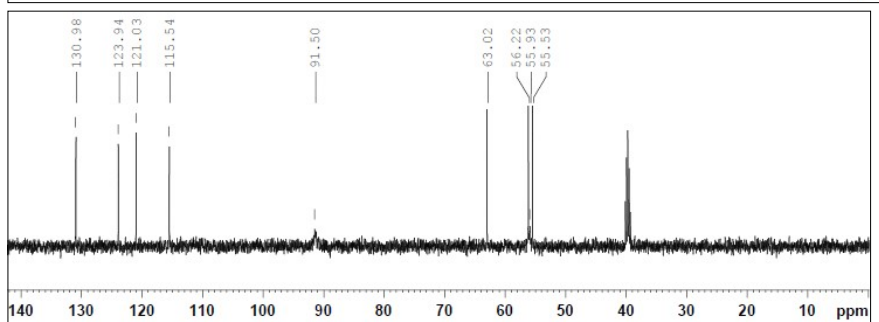
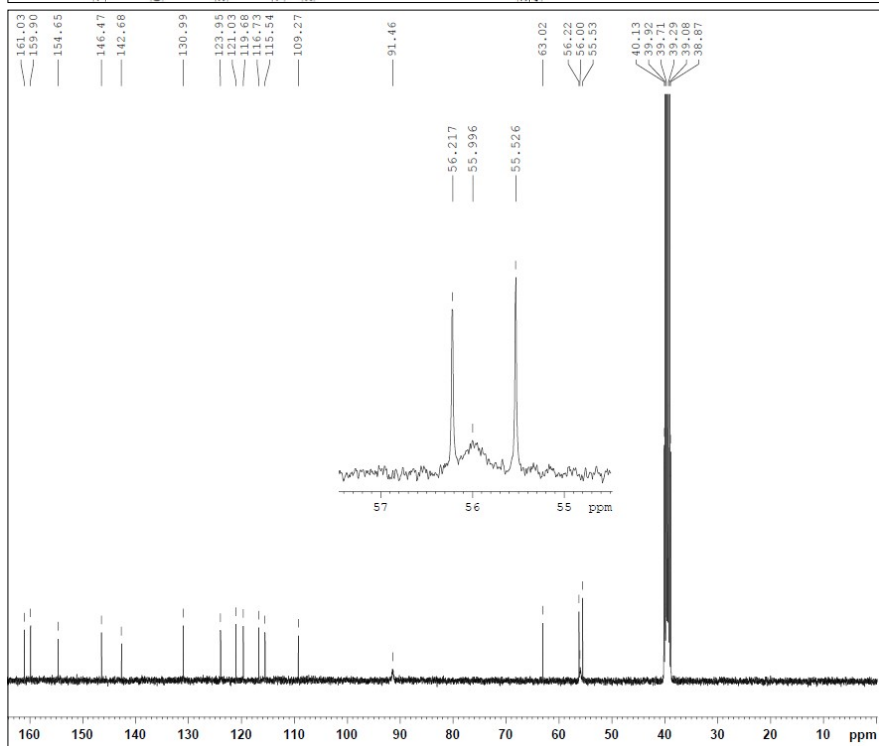
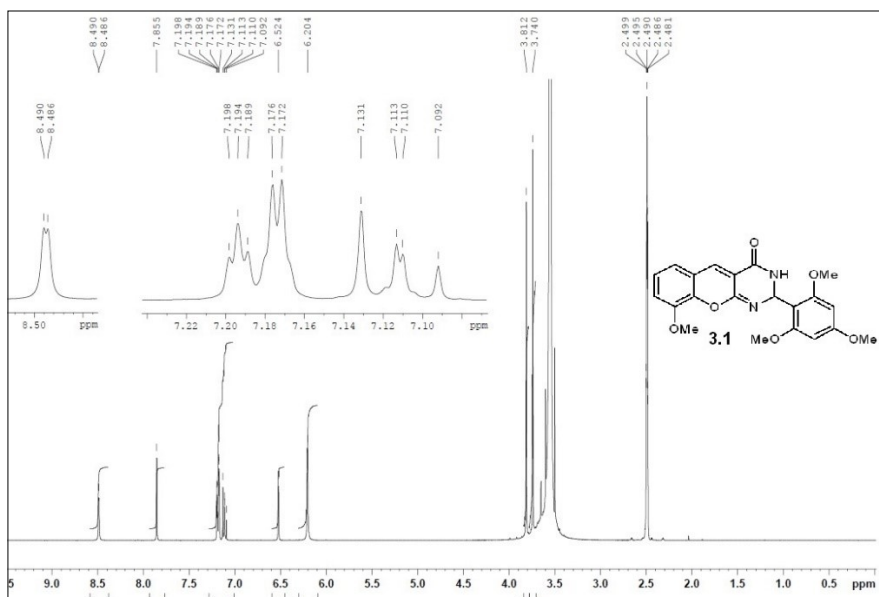
<sup>1</sup>These authors contributed equally

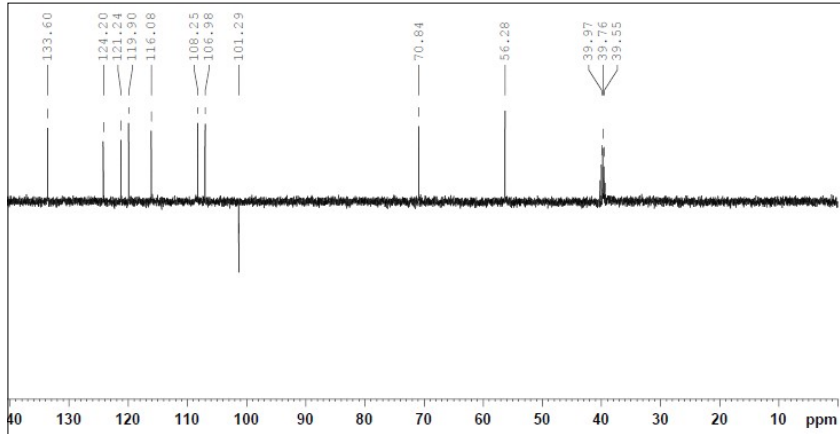
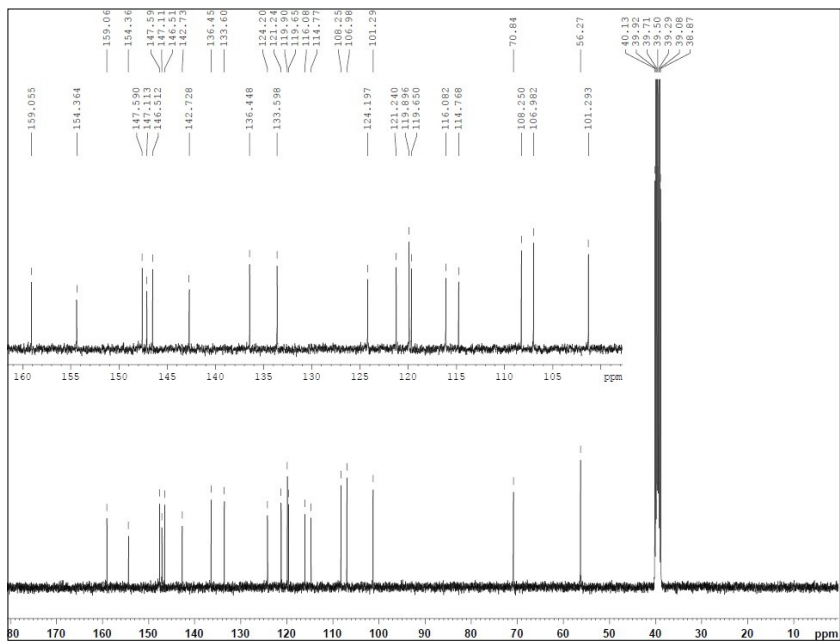
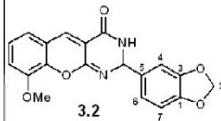
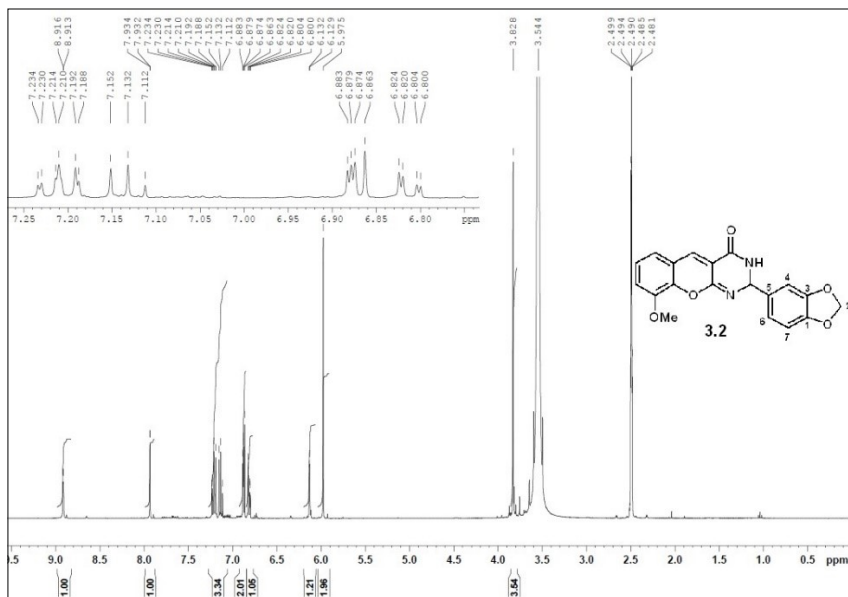
## Contents of Supporting Information

- General compound characterization data,  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT 135 NMR spectra (400 Mhz, DMSO- $d_6$ ,  $\delta$  ppm) of compounds **2.6**, **2.7**, **3.1-3.6**, **4.1-4.30**, **5**, **6**, **7** and **8**, page S3-43
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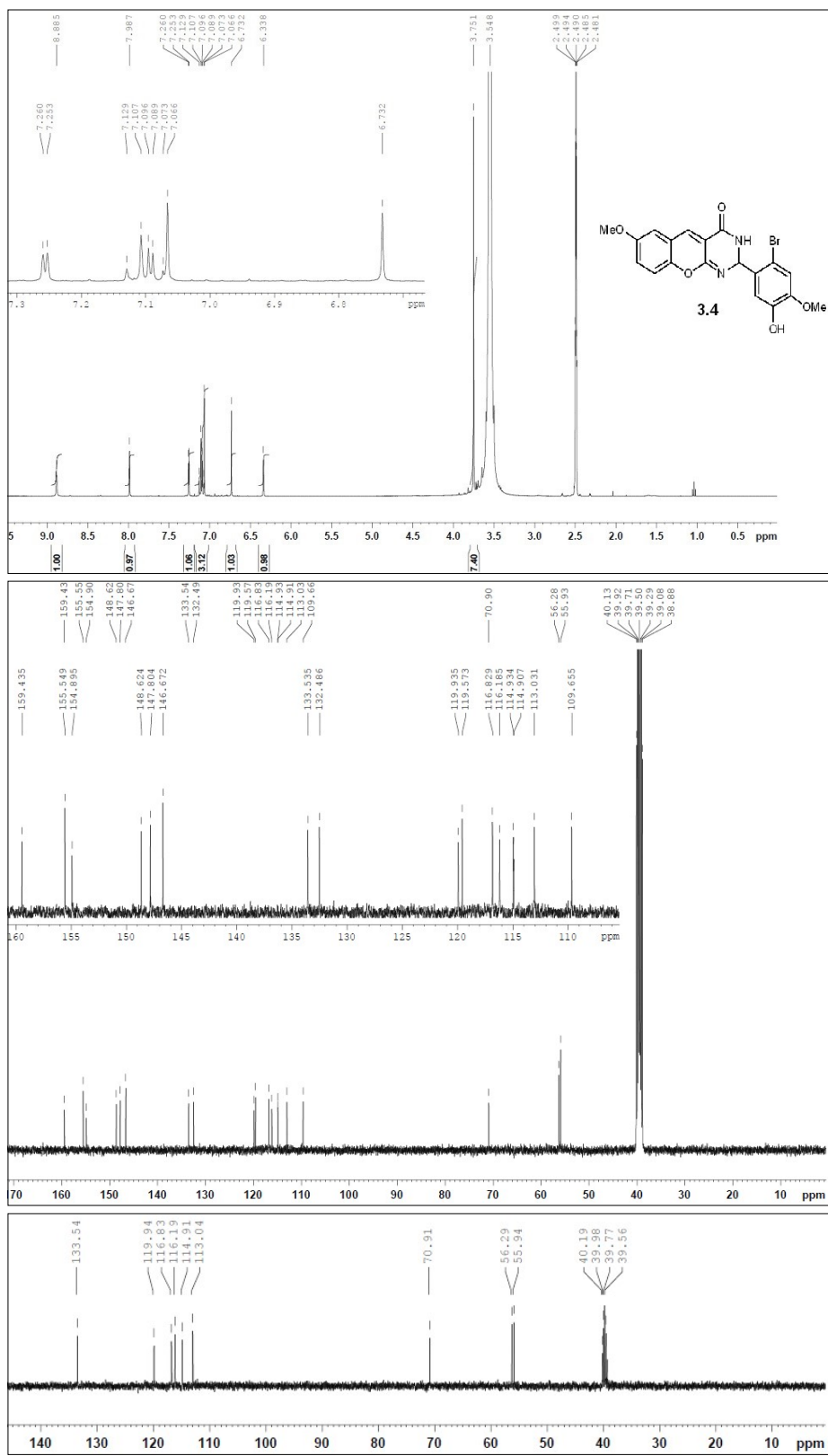






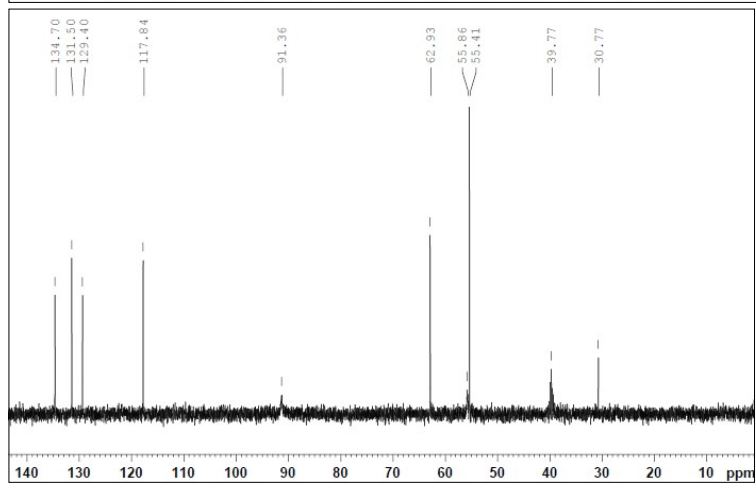
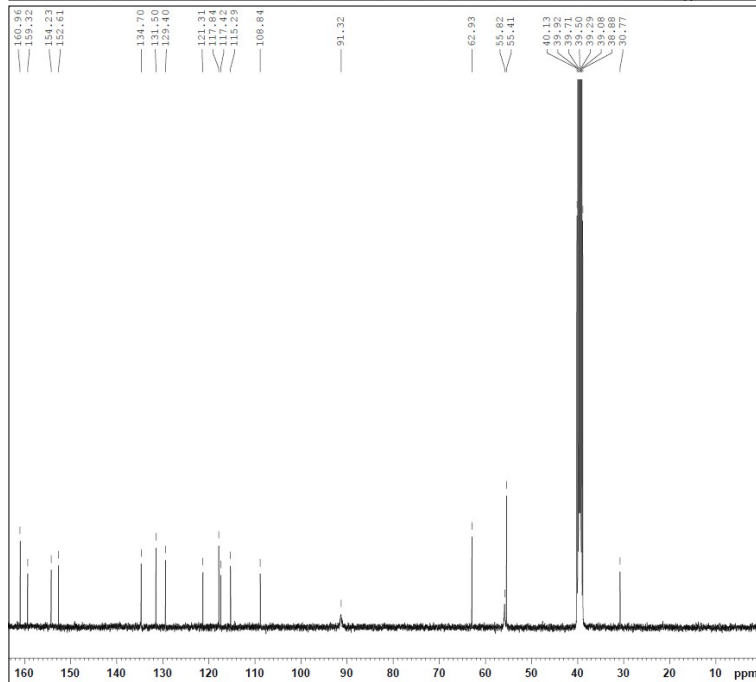
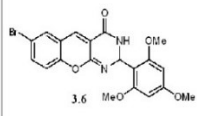
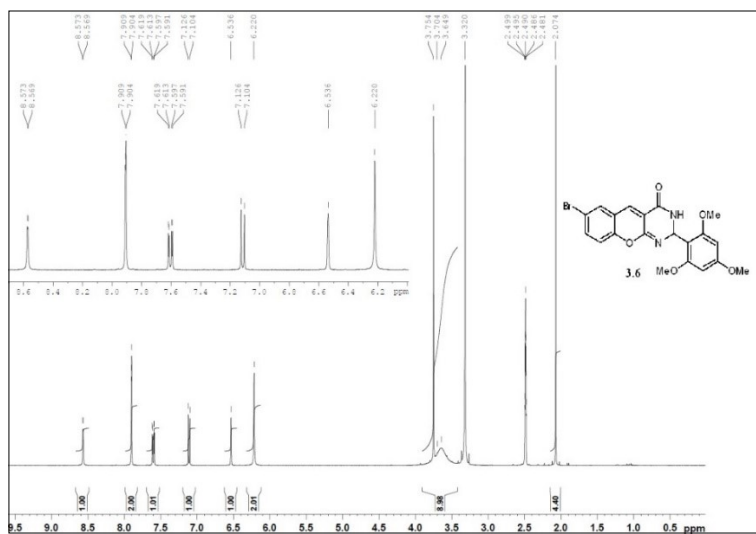


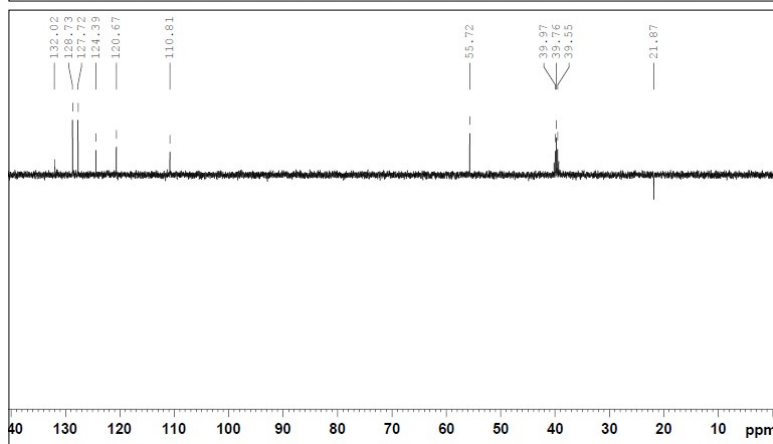
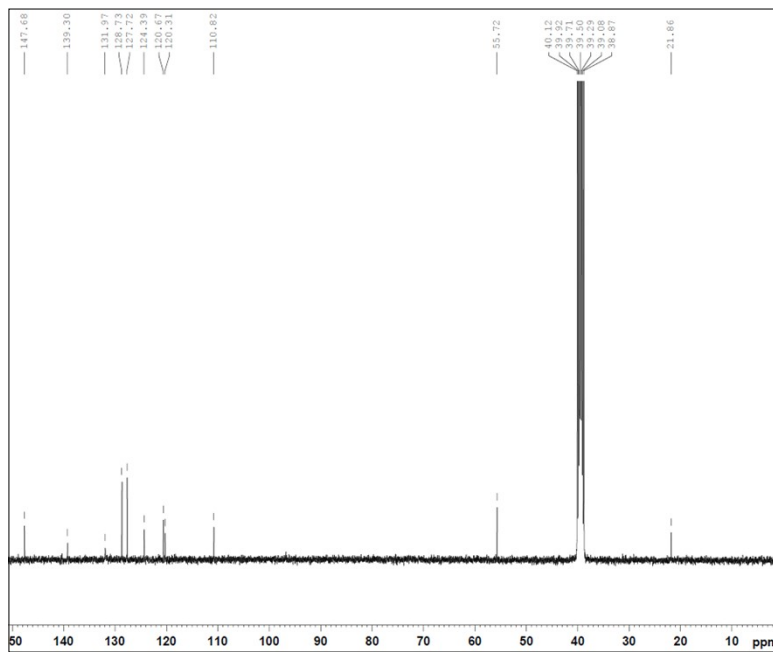
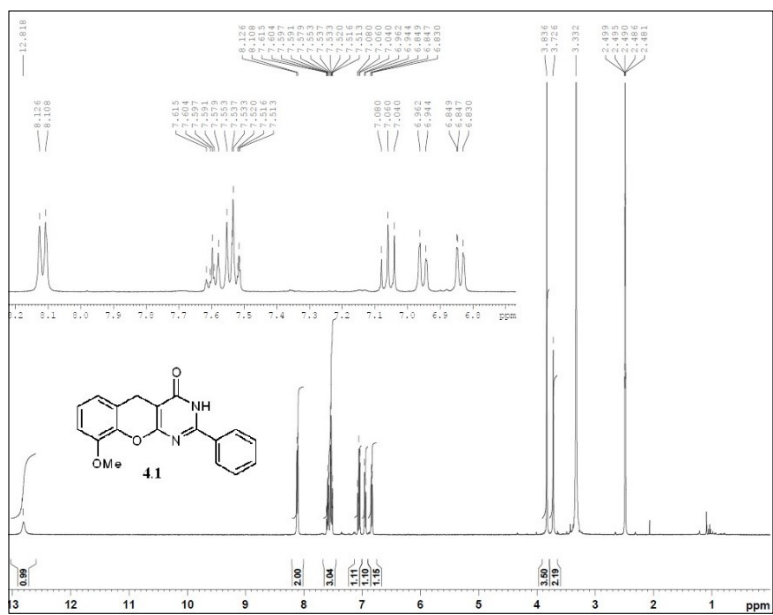


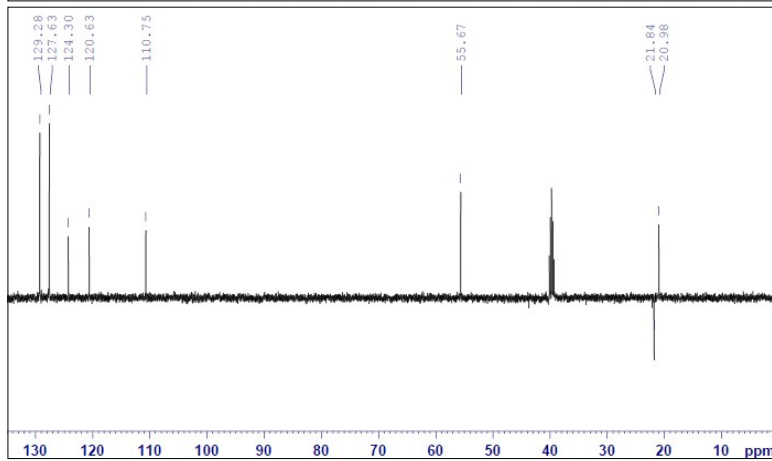
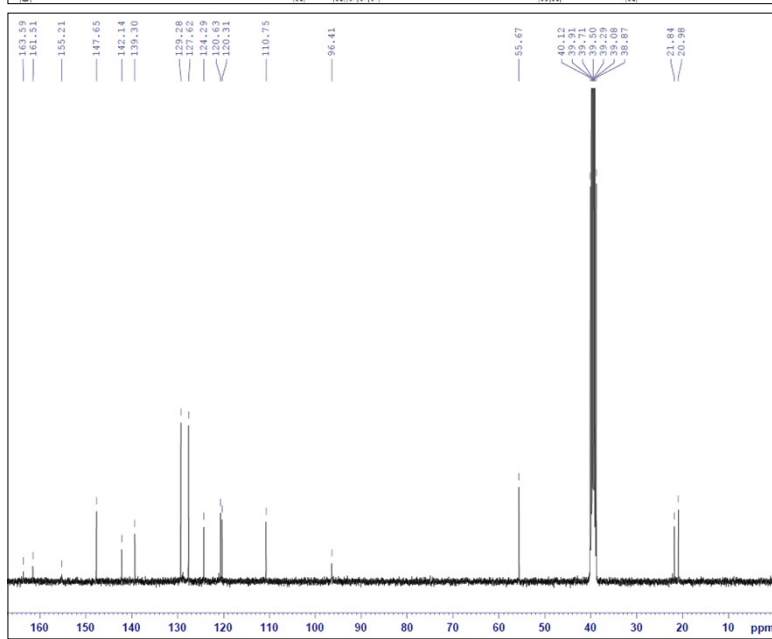
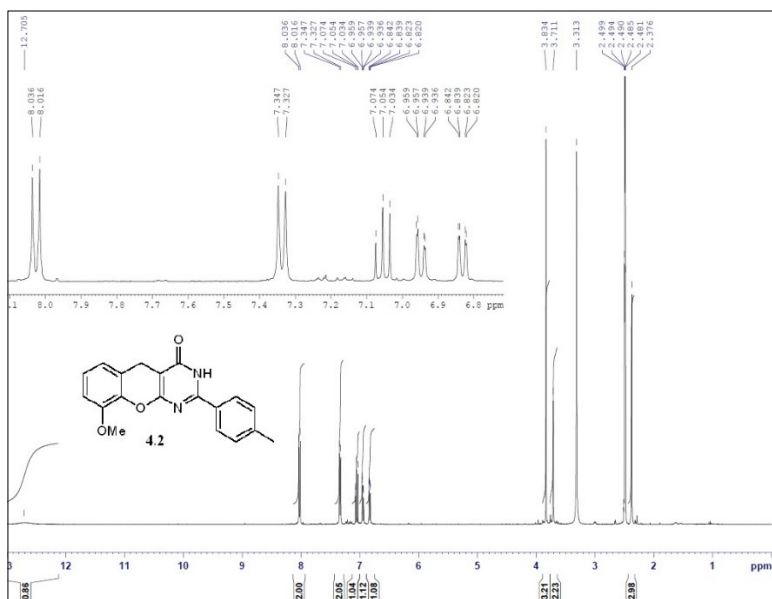






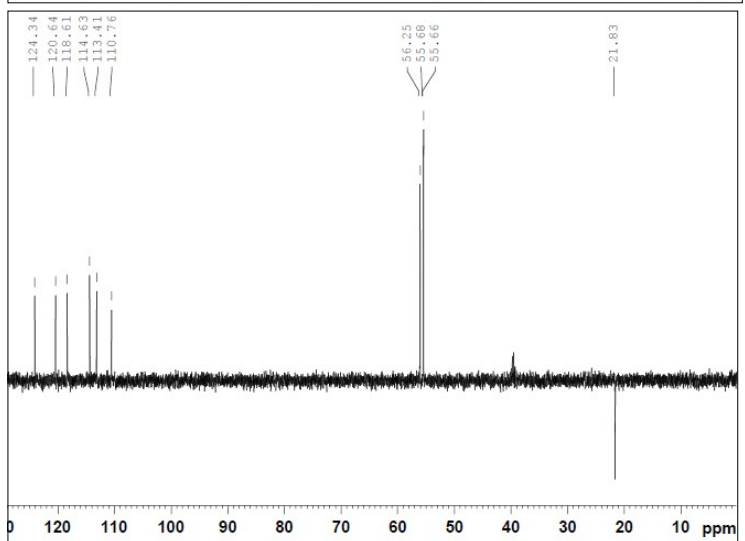
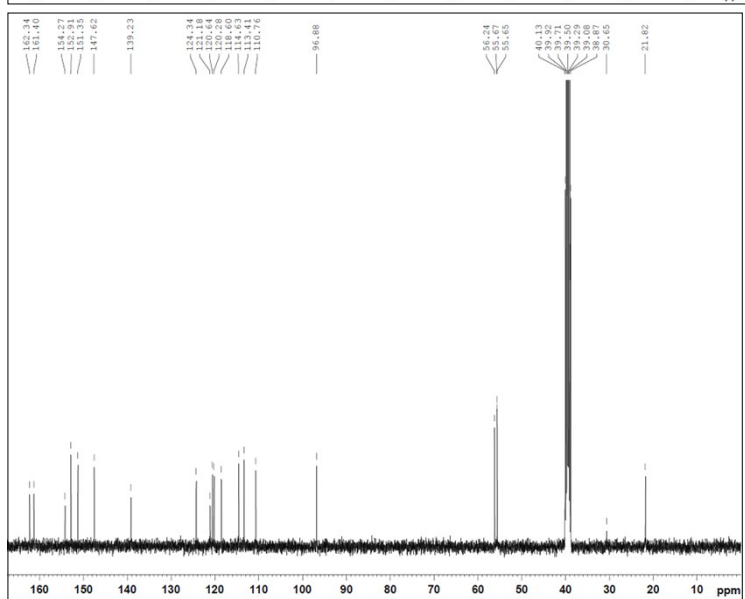
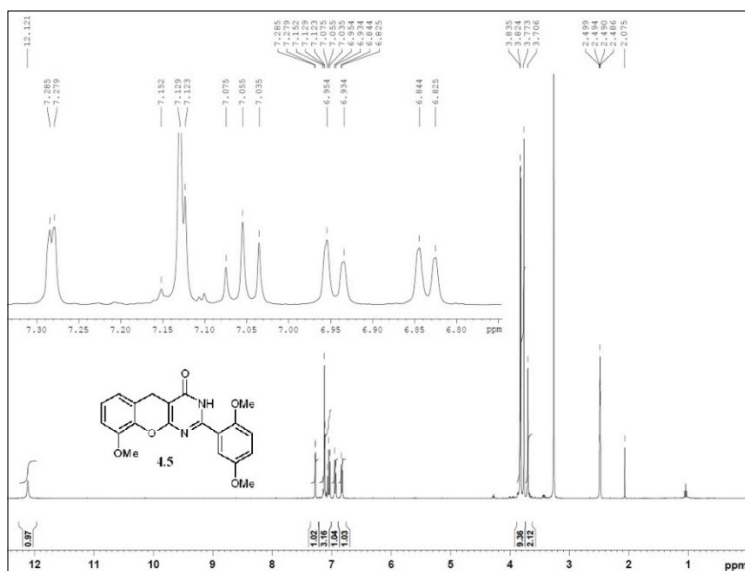


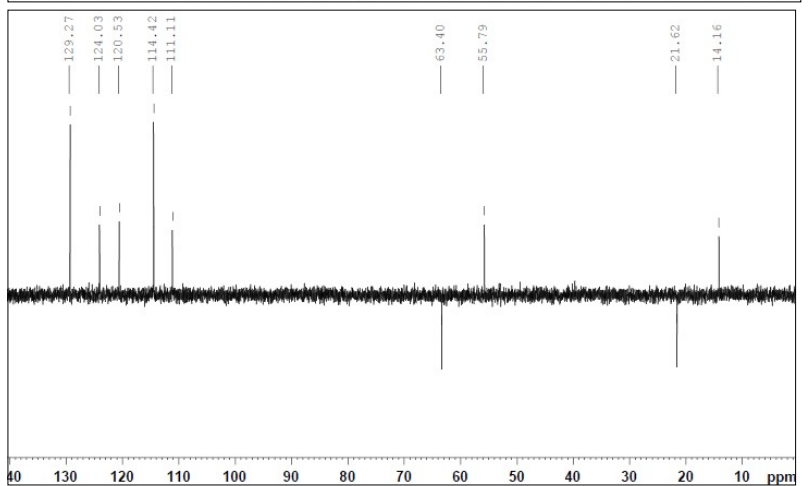
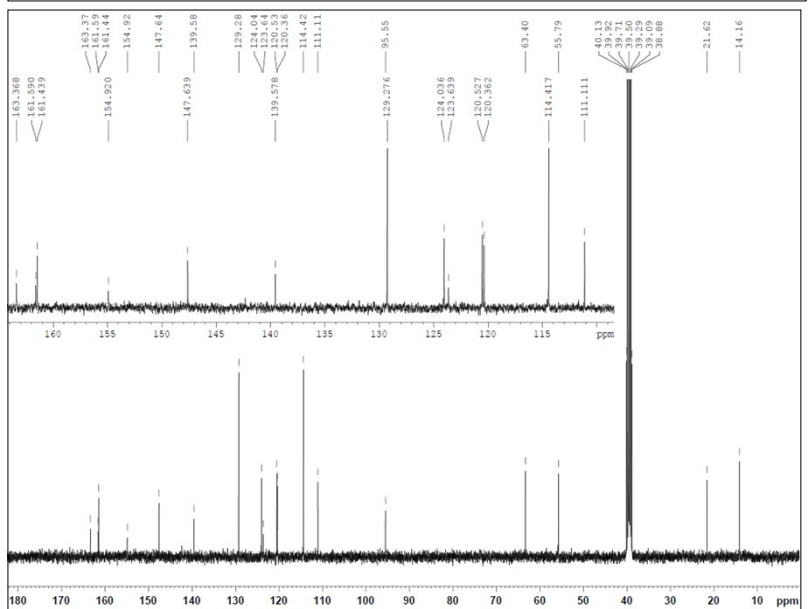
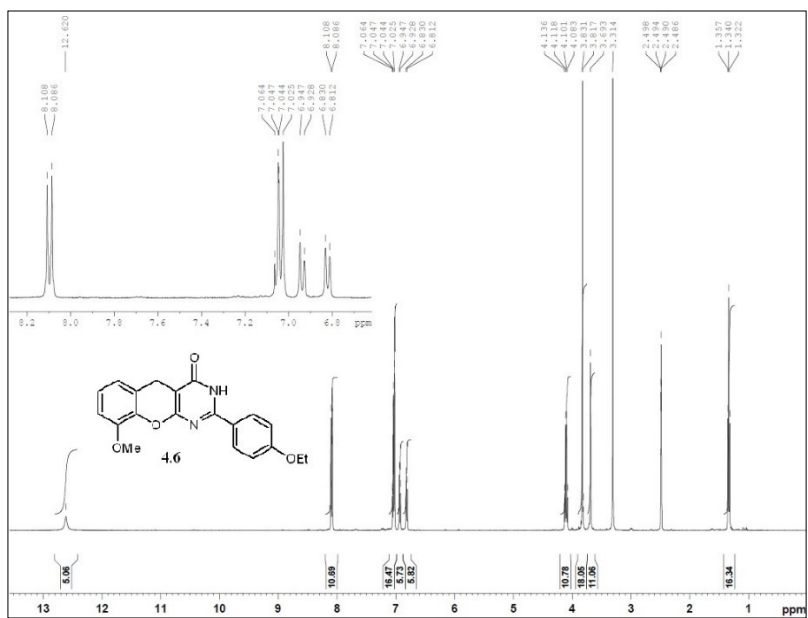




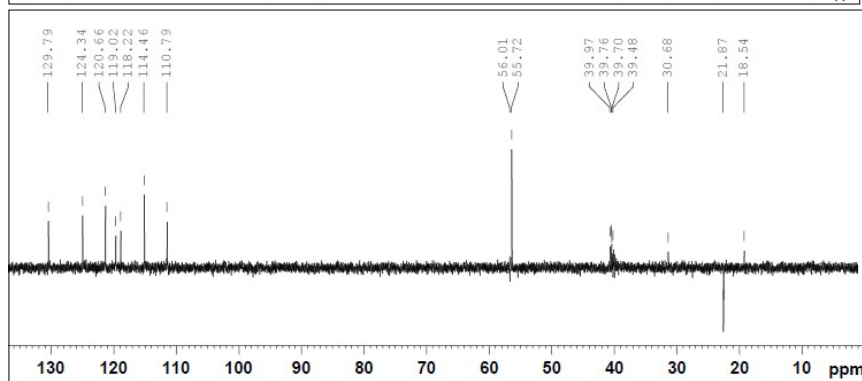
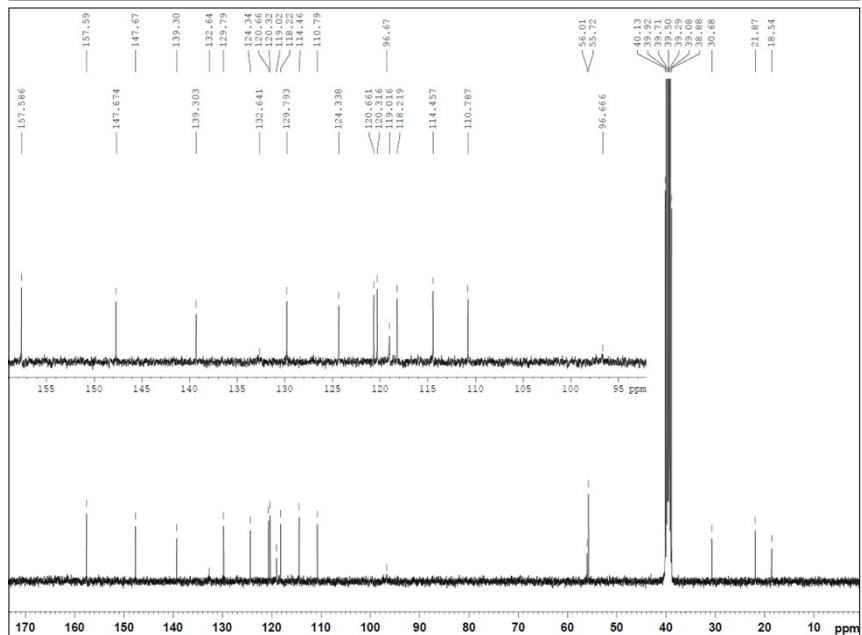
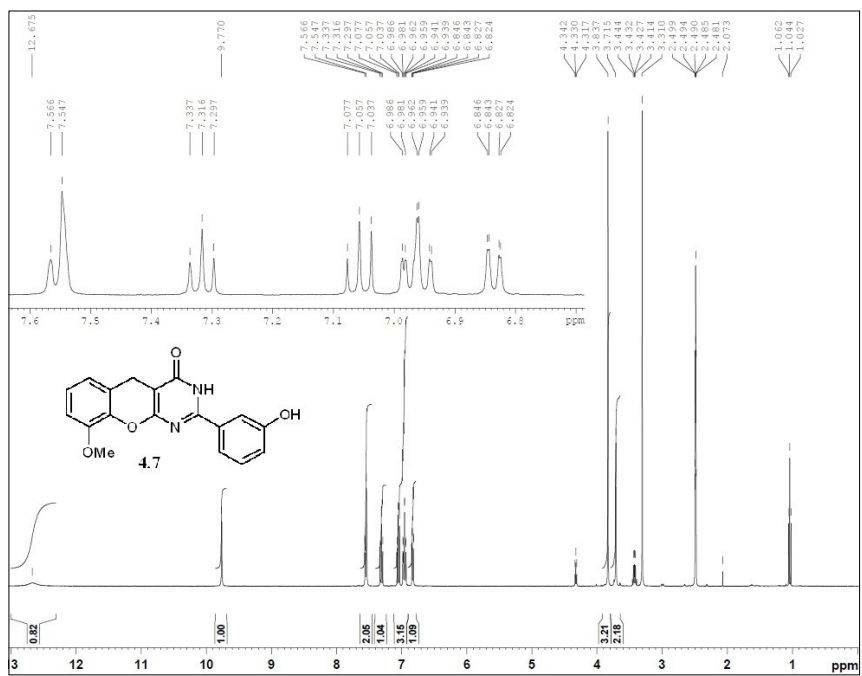


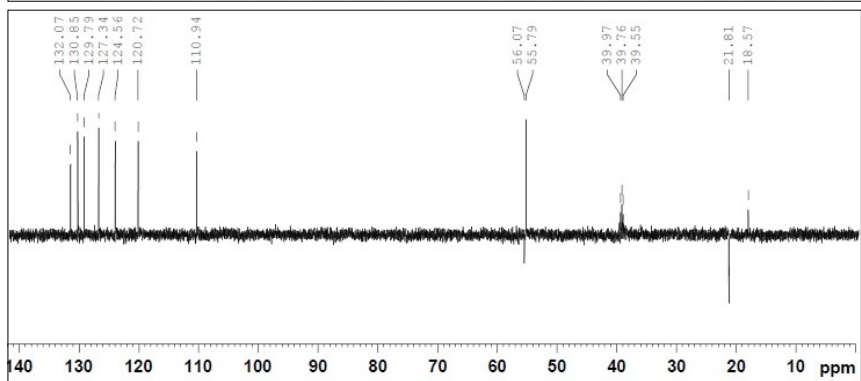
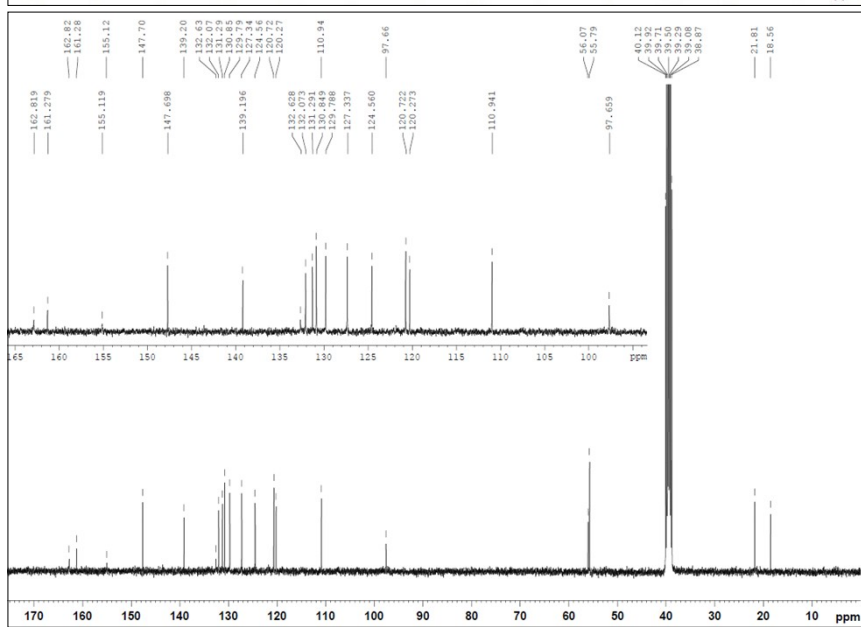
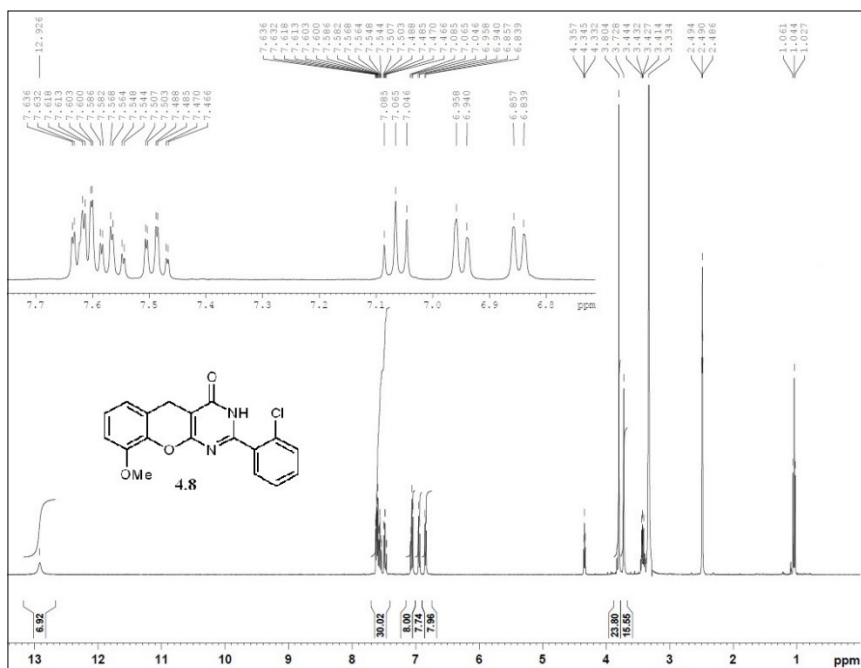


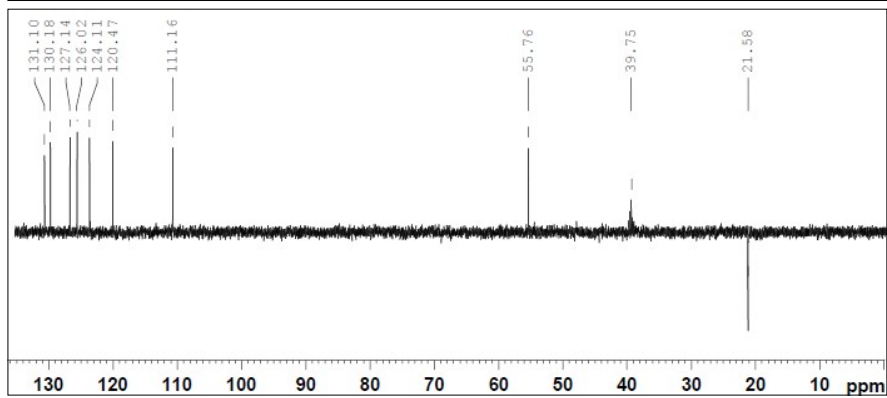
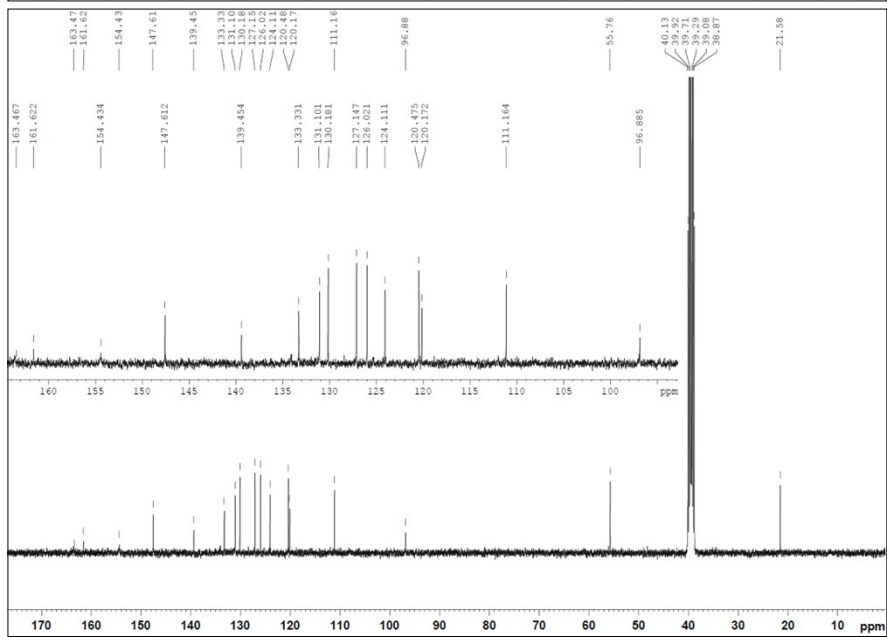
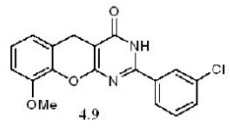
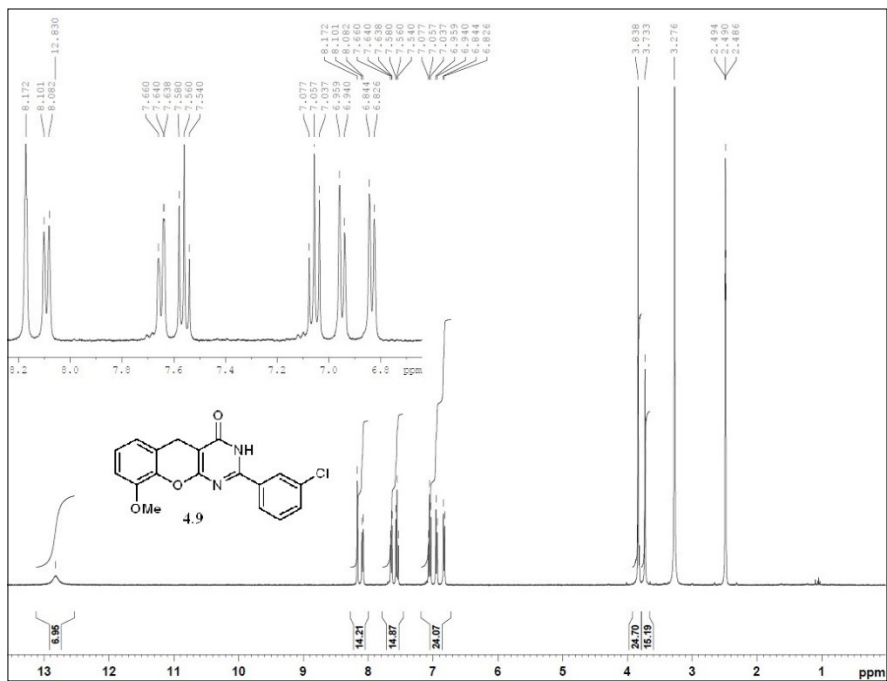




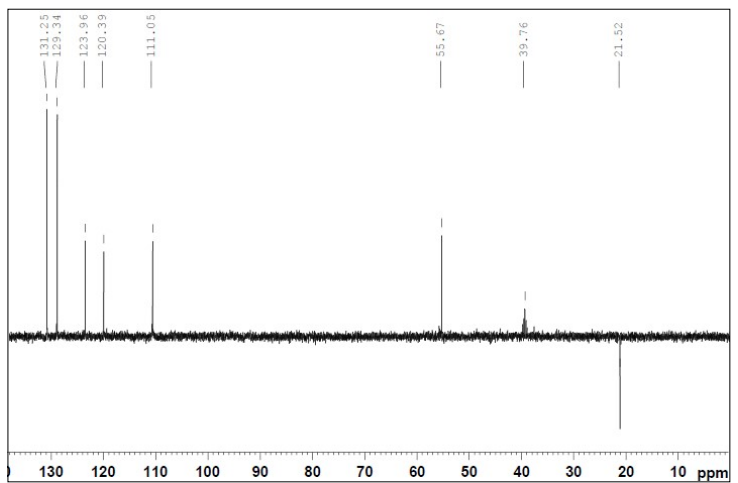
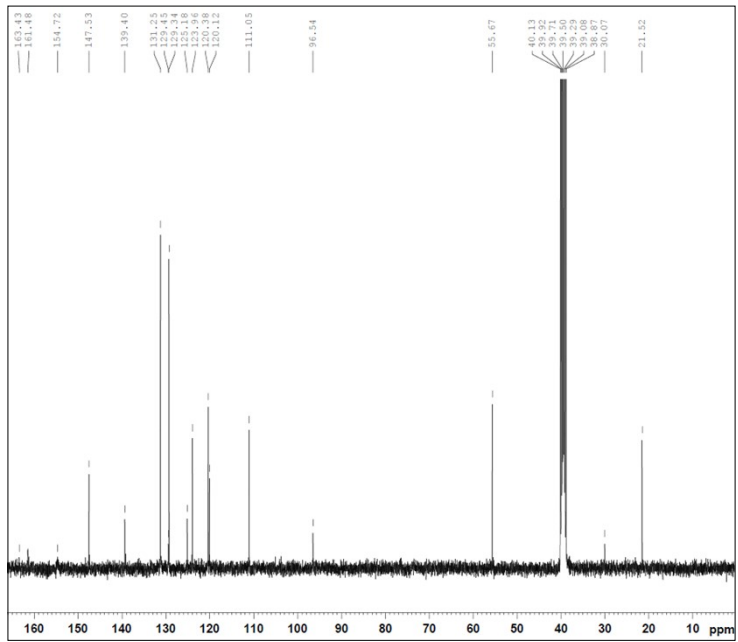
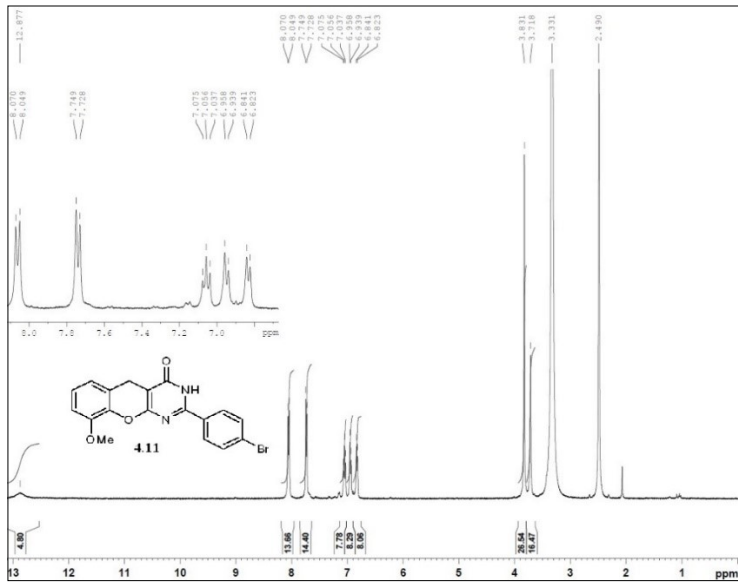














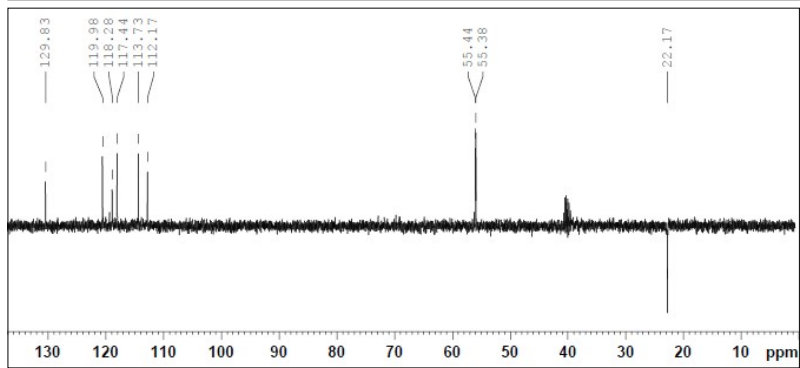
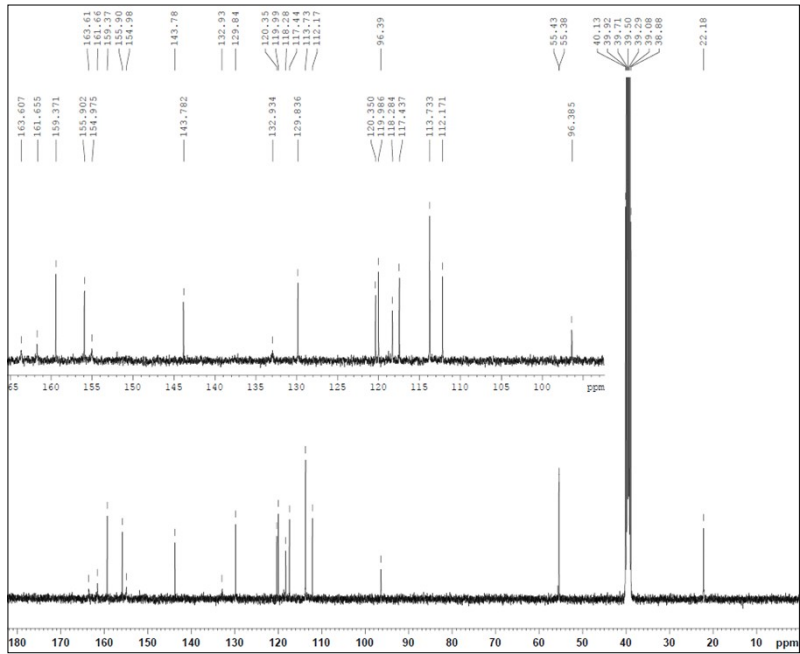
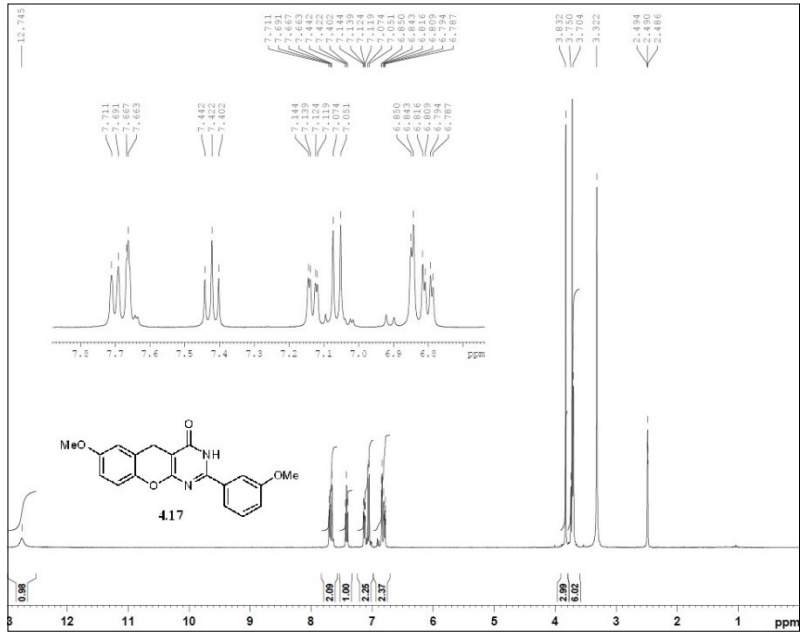


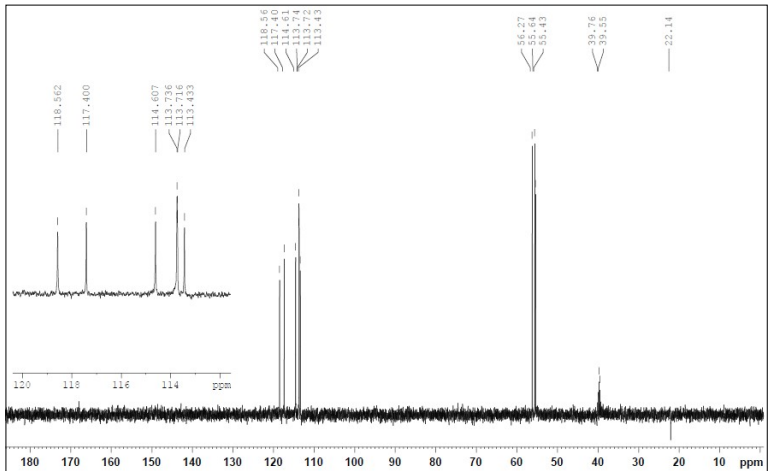
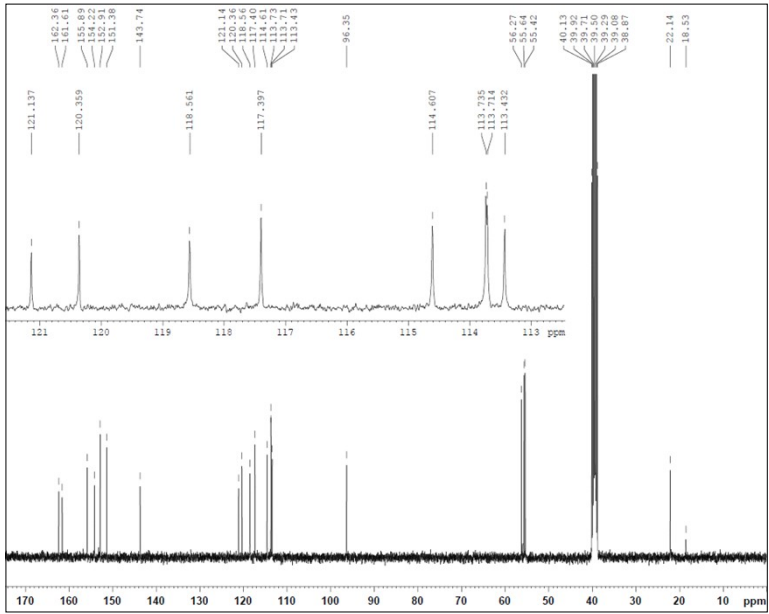
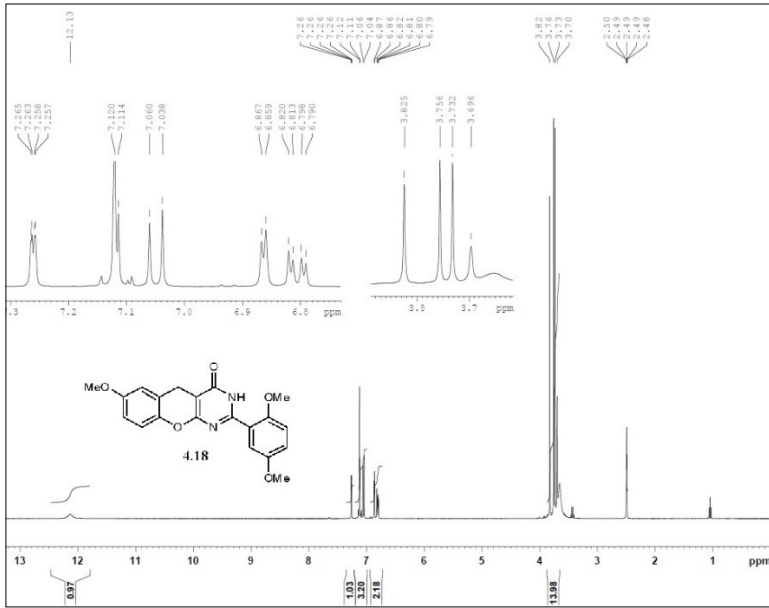


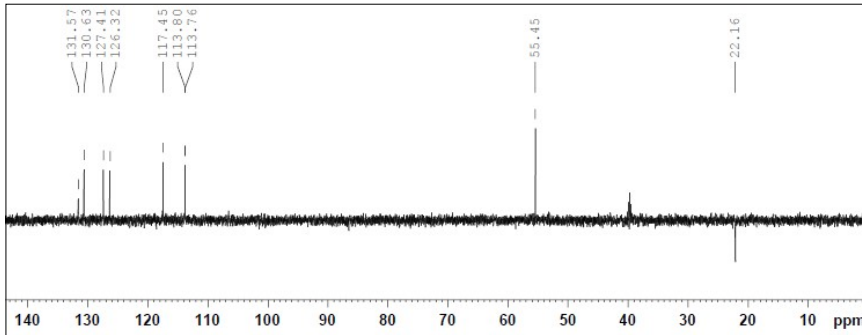
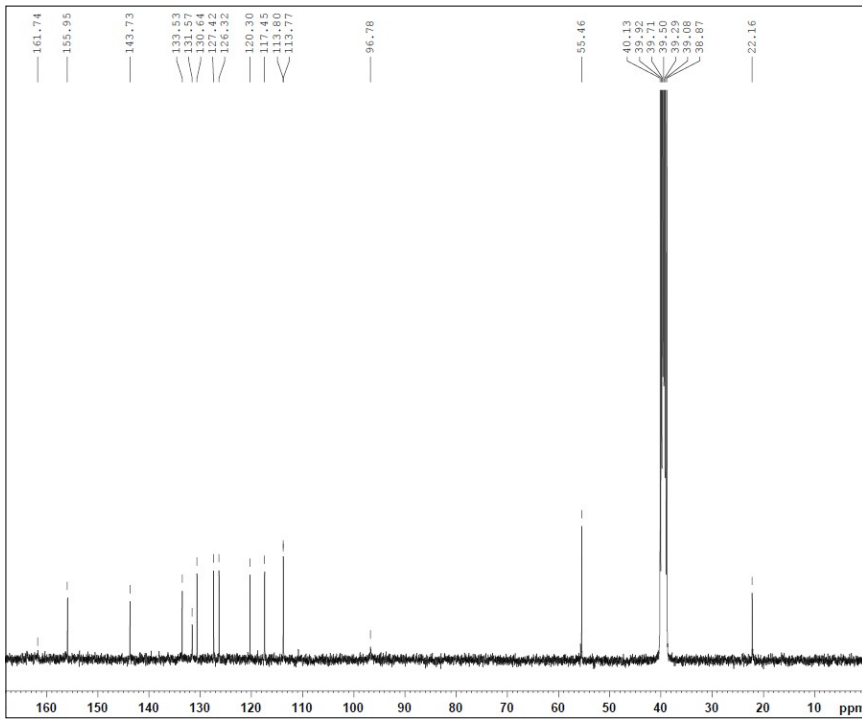
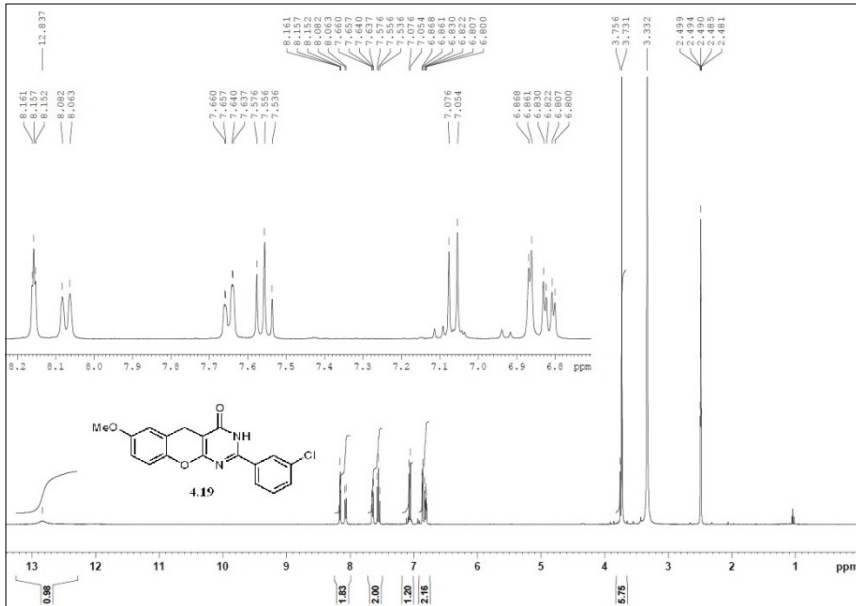




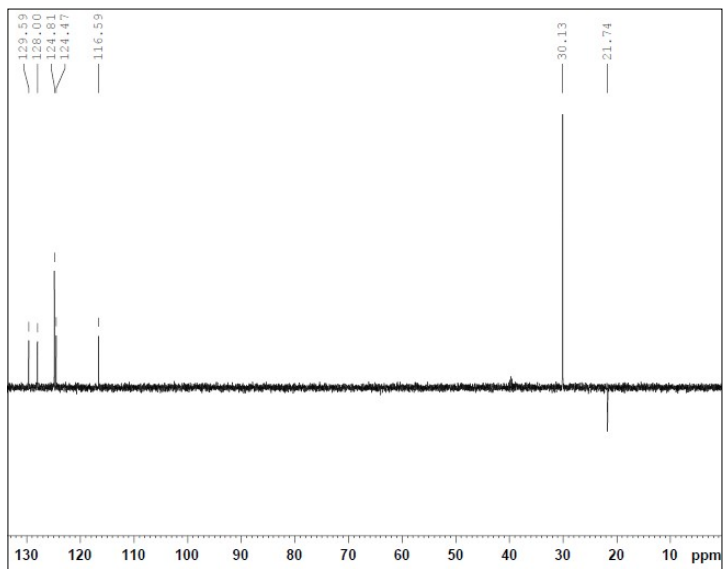
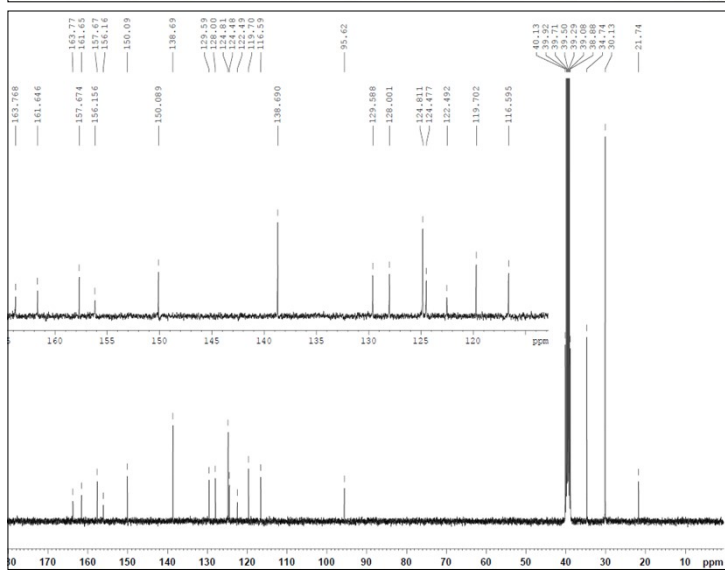
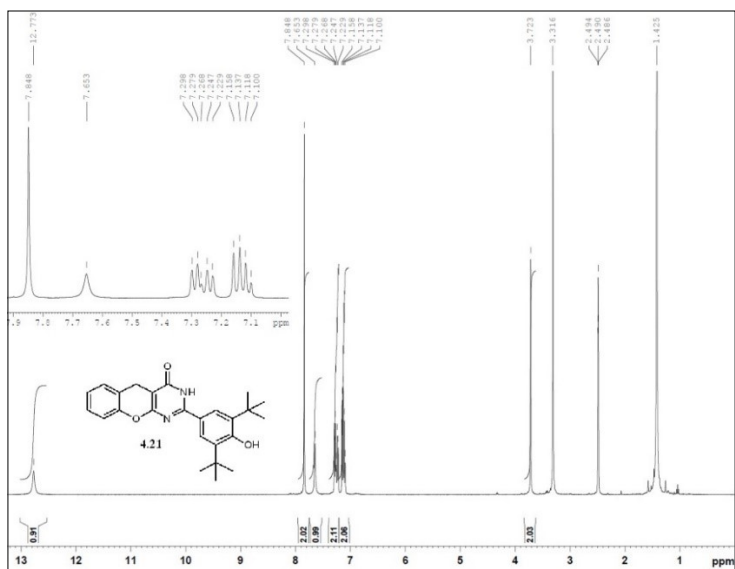








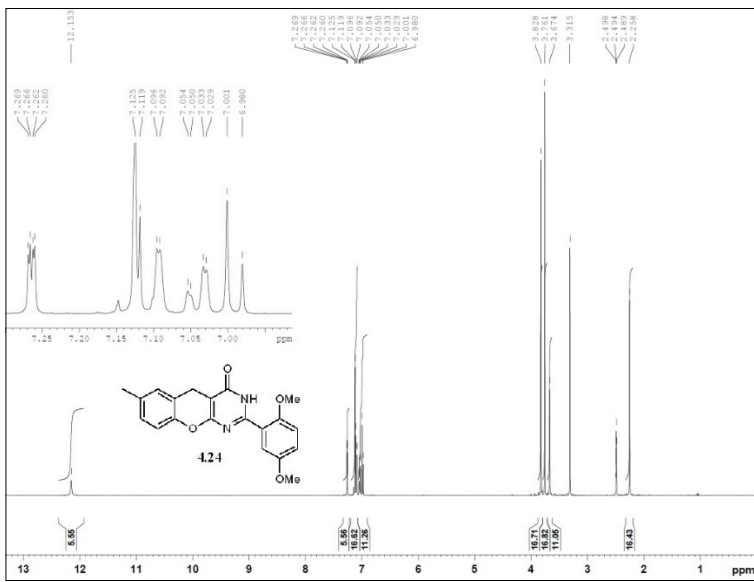
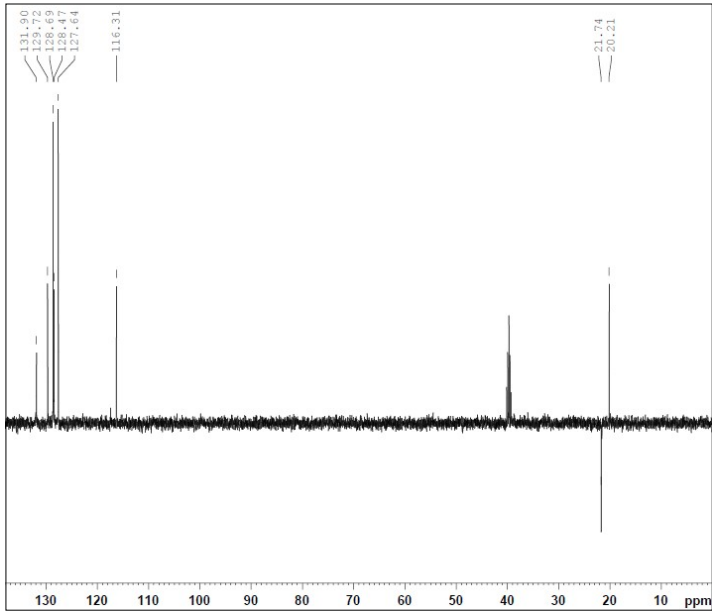




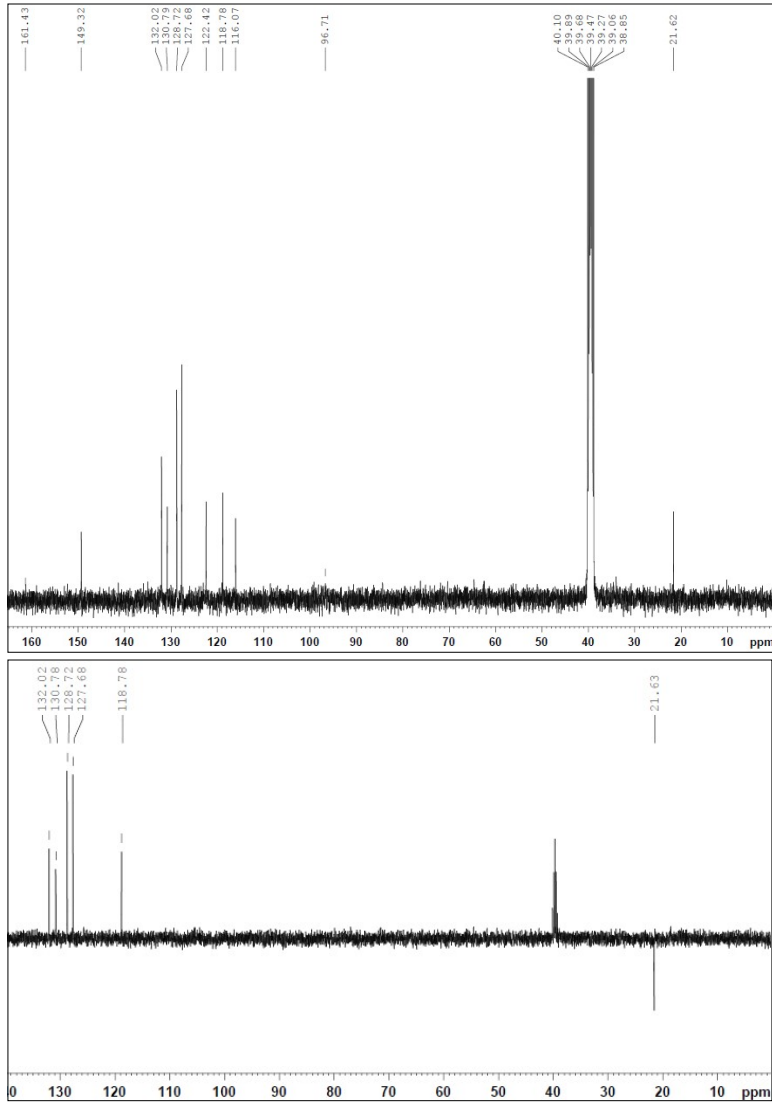






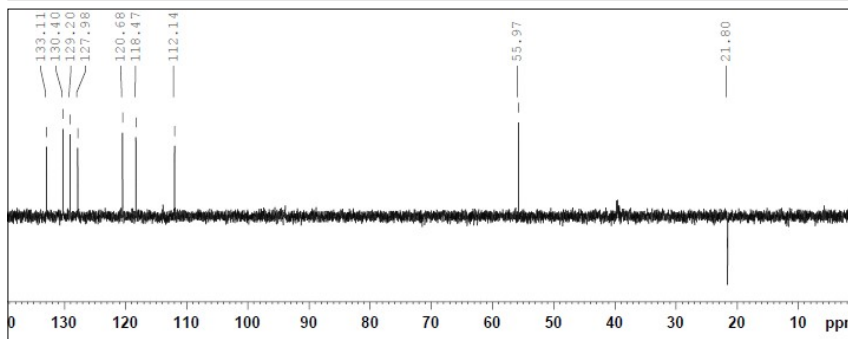
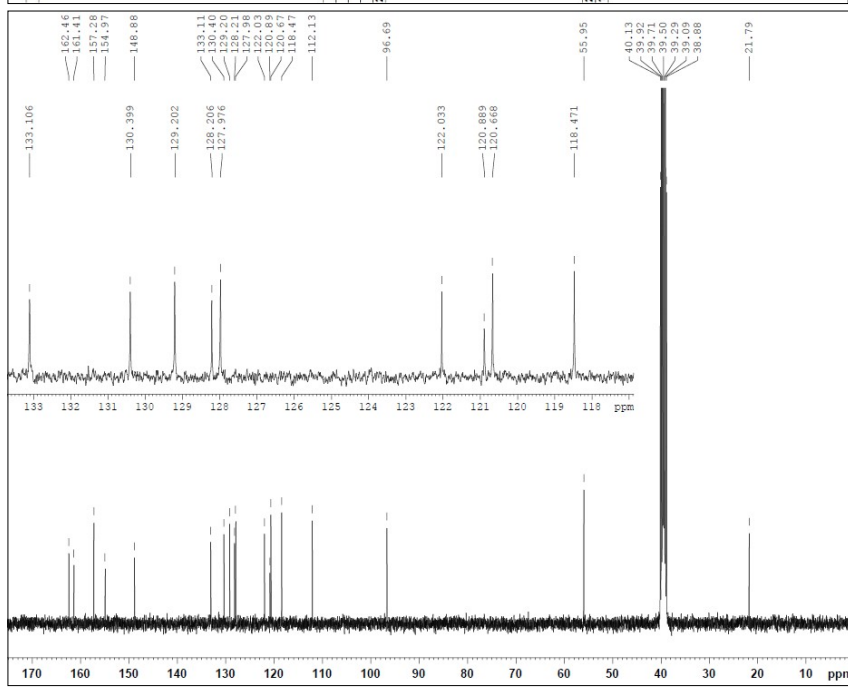
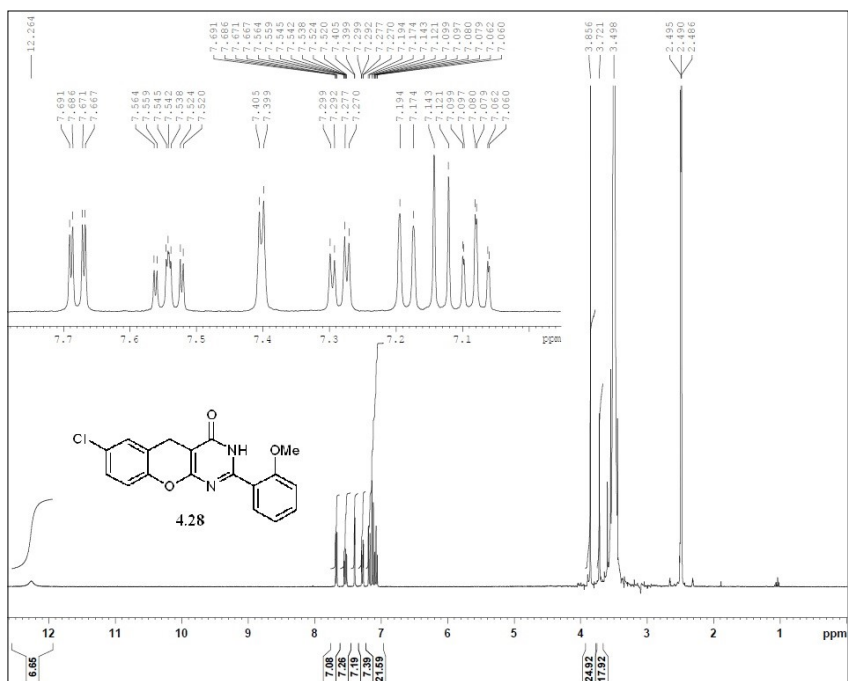






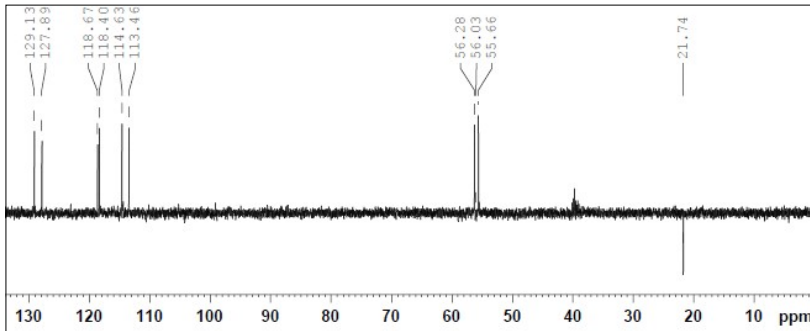
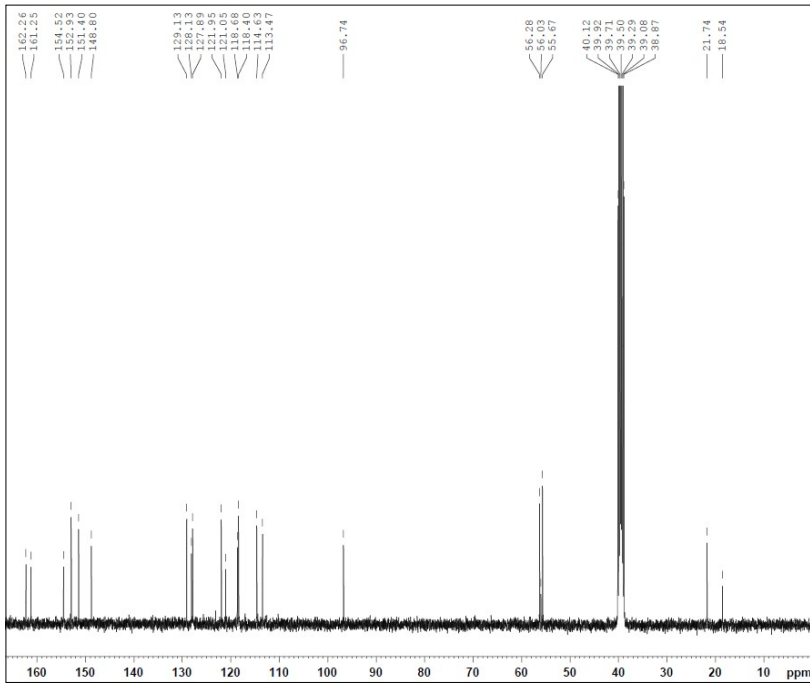
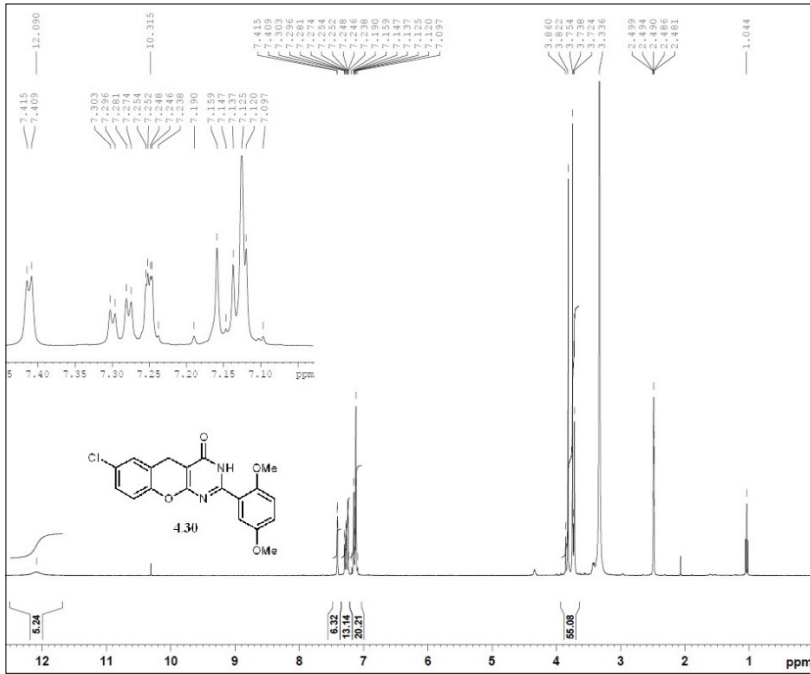
















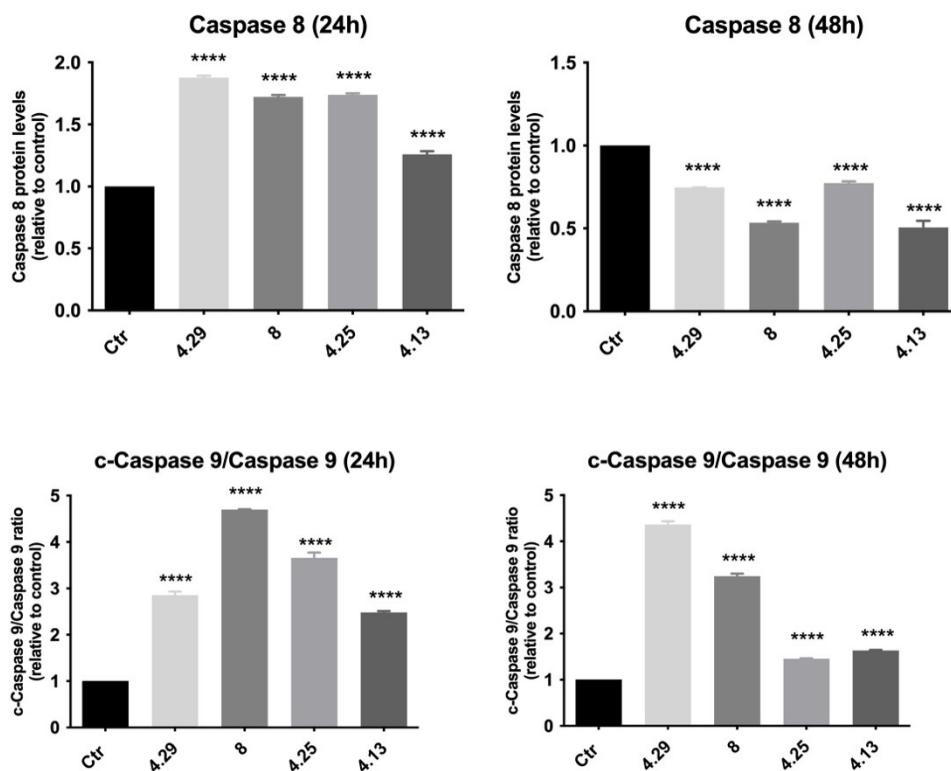


## Biology

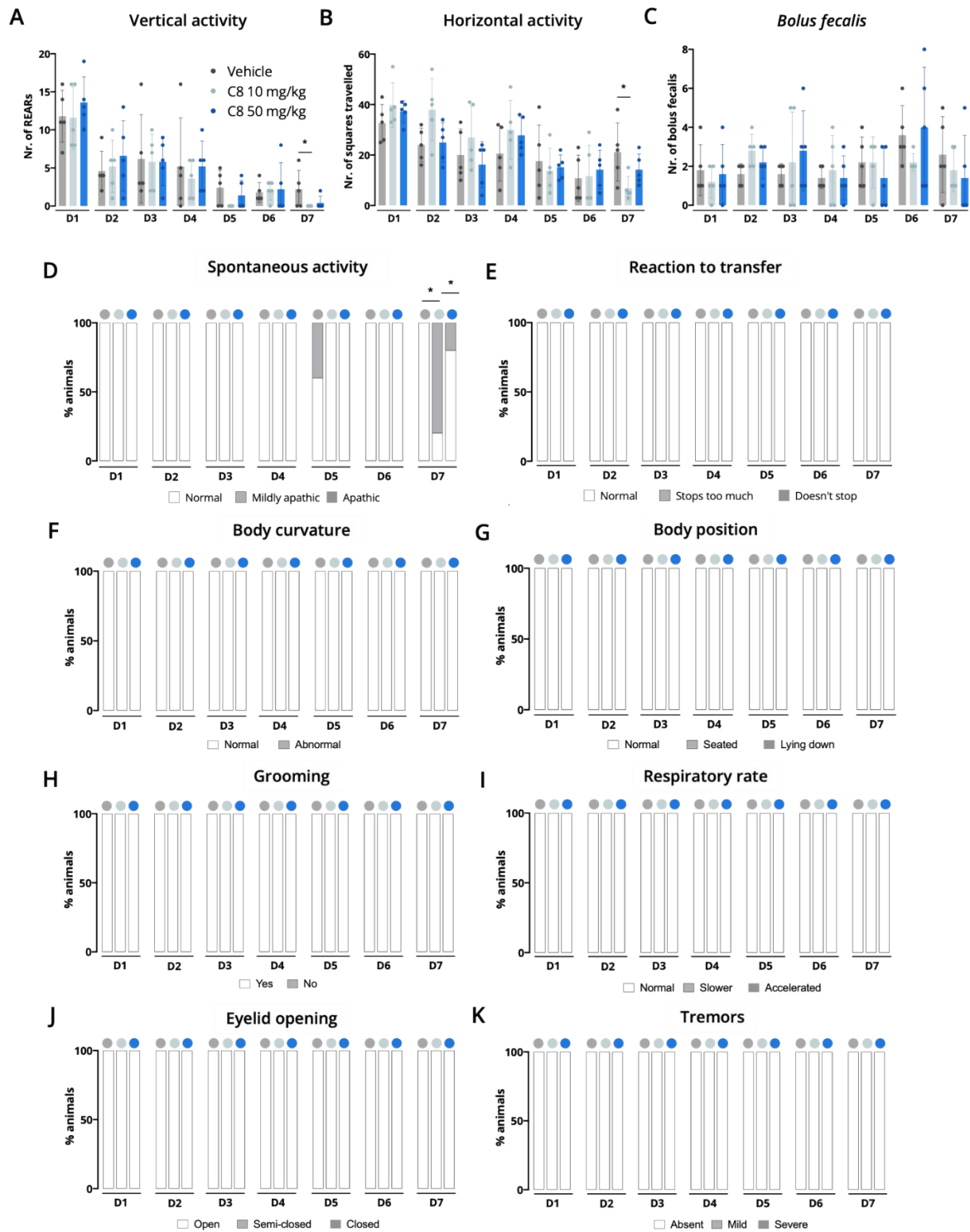
**Table S1** Cell viability results of chromene-based compounds **3** and **4** in Hs578t, MDA-MB-231 and MCF-7 breast cancer cell lines, after 72 h of treatment with two concentrations (10 and 30  $\mu$ M).

| Compounds   | Cell viability (%) |            |            |            |            |            |
|-------------|--------------------|------------|------------|------------|------------|------------|
|             | Hs578t             |            | MDA-MB-231 |            | MCF-7      |            |
|             | 10 $\mu$ M         | 30 $\mu$ M | 10 $\mu$ M | 30 $\mu$ M | 10 $\mu$ M | 30 $\mu$ M |
| <b>3.1</b>  | 94.8               | 86.0       | 83.6       | 77.4       | 99.9       | 91.8       |
| <b>3.2</b>  | 100.3              | 96.5       | 88.2       | 62.2       | 84.9       | 82.1       |
| <b>3.3</b>  | 98.4               | 83.0       | 85.8       | 64.1       | 85.9       | 84.8       |
| <b>3.5</b>  | 97.0               | 65.5       | 86.3       | 78.5       | 100.7      | 100.0      |
| <b>3.6</b>  | 95.2               | 68.6       | 80.6       | 64.4       | 90.6       | 76.2       |
| <b>4.1</b>  | 91.1               | 75.8       | 91.8       | 89.2       | 91.2       | 88.5       |
| <b>4.2</b>  | 86.8               | 63.3       | 99.2       | 91.1       | 78.5       | 77.2       |
| <b>4.3</b>  | 86.0               | 80.2       | 82.9       | 63.6       | 85.5       | 54.8       |
| <b>4.4</b>  | 99.3               | 85.3       | 70.5       | 51.6       | 93.6       | 71.3       |
| <b>4.5</b>  | 72.7               | 74.0       | 87.1       | 88.4       | 77.7       | 76.6       |
| <b>4.7</b>  | 75.9               | 67.3       | 101.6      | 101.8      | 38.0       | 25.4       |
| <b>4.8</b>  | 81.7               | 72.5       | 92.5       | 90.1       | 74.7       | 58.2       |
| <b>4.9</b>  | 72.0               | 49.9       | 80.8       | 76.9       | 69.8       | 40.4       |
| <b>4.10</b> | 66.8               | 40.6       | 92.0       | 80.8       | 78.4       | 52.4       |
| <b>4.11</b> | 97.7               | 84.4       | 83.7       | 80.4       | 83.8       | 75.3       |
| <b>4.12</b> | 97.5               | 84.4       | 105.2      | 97.8       | 88.8       | 82.9       |
| <b>4.13</b> | 30.0               | 18.7       | 68.1       | 58.6       | 59.8       | 43.5       |
| <b>4.14</b> | 99.8               | 97.9       | 99.2       | 92.5       | 84.7       | 56.7       |

|                    |      |      |      |      |      |       |
|--------------------|------|------|------|------|------|-------|
| <b>4.15</b>        | 99.8 | 90.7 | 92.8 | 85.8 | 98.9 | 100.0 |
| <b>4.16</b>        | 87.5 | 65.6 | 85.0 | 65.9 | 90.6 | 70.4  |
| <b>4.17</b>        | 88.0 | 77.0 | 69.3 | 41.2 | 73.1 | 49.8  |
| <b>4.18</b>        | 95.9 | 96.5 | 60.9 | 49.7 | 63.7 | 57.8  |
| <b>4.19</b>        | 76.0 | 74.7 | 80.7 | 44.2 | 79.3 | 45.8  |
| <b>4.20</b>        | 76.3 | 65.4 | 69.3 | 53.2 | 88.5 | 79.2  |
| <b>4.21</b>        | 80.2 | 81.0 | 95.1 | 95.2 | 97.7 | 94.7  |
| <b>4.22</b>        | 81.3 | 62.1 | 32.6 | 15.9 | 34.7 | 23.8  |
| <b>4.23</b>        | 96.0 | 73.3 | 90.1 | 65.6 | 85.8 | 73.1  |
| <b>4.25</b>        | 40.2 | 21.1 | 62.4 | 52.1 | 39.2 | 36.7  |
| <b>4.27</b>        | 86.1 | 78.5 | 85.3 | 77.8 | 83.4 | 83.1  |
| <b>4.28</b>        | 78.4 | 38.7 | 88.2 | 65.0 | 80.0 | 57.3  |
| <b>4.29</b>        | 54.7 | 33.9 | 85.1 | 18.4 | 81.5 | 35.4  |
| <b>4.30</b>        | 91.7 | 92.0 | 91.0 | 78.0 | 69.2 | 62.6  |
| <b>7</b>           | 96.1 | 89.9 | 98.0 | 96.0 | 74.6 | 37.9  |
| <b>8</b>           | 50.4 | 32.6 | 49.9 | 40.5 | 58.0 | 25.9  |
| <b>Doxorubicin</b> | 8.65 | 7.61 | 6.72 | 5.85 | 24.5 | 28.8  |



**Figure S1.** Quantification of the expression levels of Caspase 8 levels and ratio c-Caspase 9/Caspase 9, after 24 and 48 h of incubation of Hs578t cells with compounds **4.13**, **4.25**, **4.29** and **8**. Values are mean  $\pm$  SEM of 3 independent experiments. \*\*\*\* p<0.001, \*\*\*p<0.005, \*\*p<0.01, \*p<0.05 when compared with control by one-way ANOVA.



**Figure S2.** Mouse welfare evaluation tests. Overall, no differences were found between vehicle- and chromene **8** 10 mg/kg- and 50 mg/kg-treated animals in any of the tests. Only on day 7, animals treated with 10 mg/kg of chromene **8** were mildly apathic, as seen in spontaneous activity (**D**), which was further



supported by the decreased number of rears and number of squares travelled in vertical (A) and horizontal (B) exploration, respectively. One-way ANOVA with Tukey's or Dunnett T3 Post-Hoc analysis (n=5/group). All data are expressed as group mean  $\pm$  SD (\* p<0.05).

**Table S2.** Primary and secondary antibodies used in the Western blot assays.

| <b>Antibody designation</b>                   | <b>Label</b>                | <b>Reference</b> | <b>Dilution</b> |
|---|-----------------------------|------------------|-----------------|
| <b>Rabbit anti-PARP</b>                       | Cell signaling              | #9542            | 1:500 5% BSA    |
| <b>Mouse anti-Caspase 9</b>                   | Cell signaling              | #9508            | 1:1000 5% BSA   |
| <b>Mouse anti-Caspase 8</b>                   | Santa Cruz<br>Biotechnology | sc-81656         | 1:1000 5%BSA    |
| <b>Rabbit anti-Caspase 3</b>                  | Cell signaling              | #14220           | 1:1000 5% BSA   |
| <b>Rabbit anti-Bad</b>                        | Cell signaling              | #9239            | 1:1000 5%BSA    |
| <b>Rabbit anti-Bid</b>                        | Cell signaling              | #2002            | 1:500 5% BSA    |
| <b>Rabbit anti-BIM</b>                        | Cell signaling              | #2933            | 1:500 5% BSA    |
| <b>Rabbit anti-<math>\beta</math>-tubulin</b> | Abcam                       | ab6046           | 1:10000 5% BSA  |
| <b>Goat-anti-rabbit IgG-HRP</b>               | Cell signaling              | #7074            | 1:1000 5% BSA   |
| <b>Horse-anti-mouse IgG-HRP</b>               | Cell signaling              | #7076            | 1:1000 5% milk  |